

REMARKS

Paragraph [0116] has been amended to include text found in U.S. Patent No. 5,565,355 which had been incorporated by reference. A Verified Statement concerning this amendatory material accompanies this Amendment.

It is submitted that none of this amendment to the specification is new matter and its entry is requested.

Claims 2 and 4 have been amended as suggested by the Examiner. Claims 31 and 39 have been amended in a similar manner.

Claim 39 has further been amended to indicate that the cells are washed to minimize damage to the cells to tie the language of the claim to the preamble.

Claims 83, 86, 89, 92 and 116 have been amended to more clearly set forth the claimed subject matter by reversing the order of the PEG and carbohydrate source so that it is clear that the carbohydrate source is maltose and not maltose and PEG.

It is submitted that these amendments do not raise new issues, do not require additional searching, and do not constitute new matter. Accordingly, their entry is requested.

The Examiner objected to claims 2 and 4 for use of the term “a” instead of “the.” Claims 2 and 4, as well as 31 and 39, have been amended as suggested by the Examiner to obviate this objection.

The Examiner rejected claims 83, 84, 86, 87, 89, 90, 92-95, 101, 102 and 116-119 under 35 U.S.C. § 112, first paragraph for failing to comply with the written description requirement. Specifically, the Examiner contends that the following language is new matter:

“wherein the gel medium for transformation comprises a carbohydrate source that is maltose and polyethylene glycol (PEG)”;

“wherein the gel medium for transformation comprises 6% maltose and 7% PEG”;

“wherein the layer for selection further comprises abscisic acid (ABA)”;

“wherein the gel medium for selection further comprises abscisic acid (ABA)”.

Applicants note that support for the carbohydrate source and PEG in the transformation medium can be found in paragraph [0120] on page 49 of Example 6. Support for the maltose being present at 6% (60 g/L) and the PEG being present at 7% (70g/L) can also be found in this same paragraph of the specification. Thus, the use of maltose and PEG in the transformation medium and in the amounts specified is clearly supported in the specification. Consequently, claims 83, 84, 86, 87, 89, 90, 92, 93, 116 and 117 do not constitute new matter.

Applicants note that support for the selection medium containing ABA can be found in paragraphs [0047] and [0048] on pages 15-16. Paragraph [0042] on page 14 discloses that the selection can occur using a gelled medium containing the selection agent or a layer containing the selection agent over the gelled medium. Thus, the addition of ABA to a selection medium as disclosed in paragraphs [0047] and [0048] could be to either a gelled medium or a layer over the gelled medium. Thus, the disclosure of adding ABA to the selection medium includes both a gel medium and a layer over a gel medium. Specific examples of selection medium containing ABA can be found in paragraphs [0102] on page 40, [103] on pages 40-41, [0106] on pages 41-42, [0110] on page 44, [0127] on page 51 and Table 15 on page 53. Thus, the use of ABA in a selection medium, either a gel medium or a layer over a gel medium, is clearly supported in the specification. Consequently, claims 94, 95, 101, 102, 118 and 119 do not contain new matter.

In view of the above remarks, Applicants submit that the quoted language is supported in the specification and is thus not new matter. Withdrawal of this rejection is requested.

The Examiner rejected claims 1-6, 8, 9, 11-27, 29-33, 35, 36, 39, 40, 42, 43, 45 and 82-119 under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner contends that the specification does not provide enablement for a method that:

- (a) does not use a culture medium for washing that comprises inorganic salts, vitamins, amino acids, inositol, casein hydrolysate, sucrose and auxin, cytokinin or ABA or combinations of hormones;
- (b) does not include an eradication step;
- (c) does include ABA in any of the steps; and

(d) does not wash the cells between *Agrobacterium* infection step, *Agrobacterium* eradication step, selection step or growing the transformed pine cells into transformed somatic embryo step wherein the cells or embryos are collected on a support membrane placed over medium comprising the constituents.

The Examiner further contends that ABA is a required constituent for Applicants' method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus*, because the claims are broadly claiming all pines of the subgenus *Pinus* and the specification has stated that it is desired to include ABA in some of the media in the transformation of certain species of Southern yellow pines. It is submitted that the Examiner is in error with respect to this rejection. It is further submitted that the following remarks demonstrate that the specification fully enables the claimed subject matter.

The test for "enablement" is whether at the time the application was filed, one reasonably skilled in the art could make and use the claimed invention, from the disclosure in the specification coupled with information known in the art, without undue experimentation. *See, e.g., In re Wright*, 27 U.S.P.Q.2d 1510, 1514 (Fed. Cir. 1993); *Scripps Clinic & Research Foundation v. Genentech, Inc.*, 18 U.S.P.Q.2d 1001, 1006 (Fed. Cir. 1991); *See also Genentech, Inc. v. Novo Nordisk*, 42 U.S.P.Q. 2d 1001, 100 (Fed. Cir. 1997) ("[R]easonable detail" must be provided in the specification "to enable members of the public to understand and carry out the invention.") Applicants submit that the emphasis is on the claimed invention, not on all of the subject matter which may be disclosed in the specification. For the reasons that follow, Applicants submit that at the time the present application was filed, the skilled artisan would have been able to make and use the claimed invention, using the teachings in the specification coupled with information known in the art, without an undue amount of experimentation.

One technique that can be used to determine if the specification is enabling is to consider the factors set forth in *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988) include: "(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth

of the claims.” However, it is not necessary that all the *Wands* factors be reviewed to find a disclosure enabling. They are illustrative, not mandatory. *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 18 U.S.P.Q.2d 1016 (Fed. Cir.), *cert. denied* 112 S. Ct. 169 (1991). Furthermore, the necessity of some experimentation does not constitute lack of enablement as long as the experimentation is not unduly extensive. *Atlas Powder Co. v. E.E. du Pont de Nemours & Co.*, 224 U.S.P.Q. 409 (Fed. Cir. 1984).

Applicants submit that the question is not whether ABA is required in all of the media used in the claimed process, but whether the specification has enabled the subject matter set forth in the claims. Applicants submit that the specification clearly demonstrates that ABA is not required for all pines of the subgenus *Pinus*. Thus, Applicants submit that the claims are properly written without specifically requiring ABA. Applicants note that the use of ABA is not excluded from the scope of the claims, and thus can be included for those lines for which its use is beneficial. Applicants further submit that the specification provides clear guidance to a skilled artisan as to when it may be beneficial to use ABA in the claimed process. In view of this guidance in the specification, a skilled artisan can readily practice the claimed invention for all pines of the subgenus *Pinus* without undue experimentation.

With respect to the specific contentions of the Examiner, Applicants submit the following remarks. First, the liquid culture medium used for washing includes inorganic nutrients, vitamins, amino acids, a carbohydrate source. Applicants note that inositol is a vitamin and that casein hydrolysate is a source of amino acids. In addition, Applicants note that the specification teaches that any liquid culture medium can be used for washing the transformed pine cells and that hormones, ABA, inositol and casein hydrolysate are not required to be present in the liquid culture medium used for washing. The specification teaches that **any** liquid culture medium can be used to wash the transformed pine cells. *See*, page 9, paragraph [0031] and page 49, paragraph [0122]. These passages clearly teach that any liquid culture medium can be used to wash the transformed cells.

Furthermore, the specification provides two specific working examples of liquid culture media used for washing the transformed pine cells. The composition of a first liquid culture medium is set forth in Example 1 in which the wash medium is liquid DCR₄. *See*, page 23, paragraph [0063]. DCR₄ comprises inorganic nutrients, vitamins, amino acids and a carbohydrate source as shown in Table 2. DCR₄ also comprises hormones, the vitamins include inositol and the amino acids include casein hydrolysate. The DCR₄ liquid culture medium **does not** contain ABA. This liquid culture medium is used in this Example to wash transformed cells of a Southern yellow pine species, i.e., *Pinus taeda*. The composition of a second liquid culture medium is set forth in Example 6 in which the wash medium is the maintenance medium of U.S. 5,565,355 without gelling agent. *See*, page 49, paragraph [0122] in conjunction with amended paragraph [0116]. The maintenance medium of the '355 patent comprises inorganic nutrients, vitamins, amino acids and a carbohydrate source. This maintenance medium, used in a liquid form as the wash medium, **does not** contain ABA, hormones, inositol and casein hydrolysate. This liquid culture medium is used in this Example to wash a species of the genus *Pinus*, subgenus *Pinus*, i.e., *Pinus radiata*. These working examples demonstrate that ABA is not required in the washing medium for divergent species of genus *Pinus*, subgenus *Pinus*, including species of Southern yellow pines.

Finally, paragraph [0069] on page 24 indicates that the wash medium is a culture medium, i.e., it could be any culture medium, such as also disclosed in paragraph [0119] on page 48. Culture media are known in the art to contain inorganic nutrients, vitamins, amino acids and a carbohydrate source, and may optionally contain hormones. Thus, the specification clearly teaches that the liquid culture medium for washing transformed pine cells only needs to contain inorganic nutrients, vitamins, amino acids and a carbohydrate source and can be any culture medium. There is no requirement for an auxin, a cytokinin, ABA or combination of the hormones. Thus, it is submitted that the specification fully enables a wash medium that does not contain ABA, as well as not containing inositol, casein hydrolysate, an auxin, a cytokinin or combination of the hormones. Furthermore, it is submitted that the specification provides guidance should it be desired to use ABA.

Second, the claims specify that the liquid culture medium used for washing comprises inorganic nutrients, vitamins, amino acids, a carbohydrate source **and an *Agrobacterium* eradicator**. Washing transformed cells with a washing medium that includes an *Agrobacterium* eradicator is an eradication step. That is, the claims specify an *Agrobacterium* eradication step which is concomitant with the washing step. Thus, Applicants submit that the claims include a step to eradicate *Agrobacterium*. In view of the clear teachings in the specification, it is submitted that the claims in which the liquid culture medium for washing that comprises inorganic nutrients, vitamins, amino acids, a carbohydrate source and an *Agrobacterium* eradicator are fully enable because the claims include an eradication step. Furthermore, eradication can occur during the selection step as shown in Examples 3 and 5. This aspect of the invention is set forth in several dependent claims. Example 3 shows that this aspect of the invention may be beneficial for some species of pine of the subgenus *Pinus*. Thus, it is submitted that the specification fully enables the claimed method in which an eradicator is contained in the wash medium, thus imparting an eradication step to the claimed method.

Third, the specification demonstrates that ABA is not required in the steps of the method for all members of the subgenus *Pinus*, although the use of ABA in certain steps may be preferred for some members of the subgenus *Pinus*, such as the Southern yellow pines. The specification clearly teaches that ABA may be beneficial for certain species of pine of the subgenus *Pinus*, but is not necessary for all species. For example, the specification discloses that it is preferred to use ABA in the selection medium for *P. taeda* and certain hybrids as shown in Examples 7 and 8. These Examples demonstrate that ABA may be preferred in other media, but is not required, because it gave either neutral results or beneficial results. However, Example 6 demonstrates that ABA does not need to be present in the selection medium, let alone any of the other media, for *P. radiata*. In addition, paragraphs [0047] and [0048] on page 16, the latter specifically noted by the Examiner, only teaches that the presence of ABA is useful for certain species of pine of the subgenus *Pinus*, namely the Southern yellow pines, and does not teach that ABA is required for all members of the subgenus *Pinus*. The specification provides clear guidance to a skilled artisan for the use of ABA in selection medium, as well as in other media for certain members of the subgenus *Pinus*. Namely,

the specification provides clear guidance that the use of ABA is preferred, especially for those species of pine of the subgenus *Pinus* which have low rates of recovery of viable transformed cells using media without the ABA. If a low rate of recovery of viable transformed cells is obtained for a member of the subgenus *Pinus*, the specification guides the skilled artisan to add ABA to certain of the medium used in the process. Thus, it is submitted that the specification fully enables the claimed method which does not require the use of ABA in the selection medium or in any other media because it provides guidance to the skilled artisan when to use ABA in such media.

Fourth, the specification clearly teaches that support membranes are not required for each of the steps of the method, although their use may be preferred. For example, Example 4 shows that the use of support membranes is not required for the selection of transformed pine cells. *See*, paragraph [0107] on page 42 which clearly shows either the non-use of support membranes or the use of support membranes. This passage teaches that it is not necessary to use support membranes, although it may be beneficial. In addition, Example 6 shows that support membranes are not required, although preferred for ease of manipulation, for preparing plants of the species *P. radiata*. Thus, it is submitted that the specification fully enables the claimed invention which does not require the use of support membranes because it provides guidance as to when it may be desired to use such support membranes.

Fifth, the Examples show that washings are not required between each of the steps, i.e., none of the Examples describe any washing steps other than the washing after the co-cultivation of pine cells with *Agrobacterium* in the manner to minimize damage to the transformed pine cells. Thus, it is submitted that the specification fully enables the claimed invention which does not have washing steps between each step of the method set forth in the claims.

Sixth, Applicants note that paragraph [0118] on page 48 teaches that any nutrient media that is commonly used for *Pinus* somatic embryogenesis is suitable for use in the method of the invention. The specification has demonstrated the use of different nutrient media in the method of the invention. Thus, it is submitted that the specification fully enables the use of various nutrient media in the claimed method.

It is evident that a proper application of the Wands factors to the subject matter of the claims readily demonstrates that the specification fully enables the claimed invention.

(1) *quantity of experimentation necessary*: The specification sets forth specific media that can be used to regenerate transformed hard pine and discloses that any medium that can be used to regenerate hard pines can be used to regenerate transformed hard pines using the techniques disclosed in the specification and set forth in the claims. The specification discloses when it is preferred to use ABA in any of the media, which in essence provides a simple screening technique. If the transformed hard pine has a low rate of recovery of viable transformed cells using media without the ABA, then ABA is preferred to be included. This screening does not represent a large quantity of experimentation, i.e., undue experimentation is not required.

(2) *the amount or direction of guidance presented*: As described above in great detail, the specification provides a very high level of guidance for making and using the claimed invention which would not require extensive, i.e., undue, experimentation. For example, the specification clearly teaches that any medium that can be used to regenerate hard pines can be used to regenerate transformed hard pines. The specification provides guidance that ABA should be included in various media if the species of transformed pine has a low rate of recovery of viable transformed cells using media without the ABA. If a low rate of recovery of viable transformed cells is obtained for a member of the subgenus *Pinus*, the specification guides the skilled artisan to add ABA to certain of the medium used in the process. Thus, it is submitted that the specification fully enables the claimed method which does not require the use of ABA in the selection medium or in any other media because it provides guidance to the skilled artisan when to use ABA in such media.

(3) *the presence or absence of working examples*: The specification contains eight working examples which provide an extensive description of the process that is used to regenerate transgenic plants of pine of the genus *Pinus* subgenus *Pinus*. These examples show the regeneration of the pines *P. radiata* and *P. taeda*, as well as elite lines of the latter. *P. taeda* is a Southern yellow pine. These working examples show that ABA is not required in the media for regenerating all of pines of the included group, show that ABA in certain media is beneficial for certain pines, such as the

Southern yellow pines. The working examples further disclose how to determine whether ABA should be included in certain media for any give pine of the genus *Pinus* subgenus *Pinus*.

(4) *the nature of the invention*: The present invention is in the area of regeneration of transformed hard pine, i.e., pine of the genus *Pinus* subgenus *Pinus*. Although regeneration of transformed hard pines has previously proved difficult, it was known that hard pines can be regenerated in tissue culture. The present invention discloses a process to regenerated transformed hard pines and discloses that any media that can be used to regenerate hard pines can be used to regenerate transformed hard pines using the disclosed process. Furthermore, the specification clearly provides a high level of enablement through the working examples which demonstrate the practice of the claimed invention.

(5) *the state of the art*: Processes for regenerating hard pines was known as evidenced by the some of the prior art cited by the Examiner. These processes were known to be useful for regenerating all species of hard pines. However, these processes were generally not sufficient for the efficient regeneration of transformed hard pines, which is the reason for the present invention. Nevertheless, the prior art processes demonstrate the broad applicability of the processes to hard pines.

(6) *the relative skill of those in the art*: The level of skill in the art is quite high as evidenced by the workers in the field, namely researchers with Ph.D. degrees or graduate students and post-docs with many years of research experience.

(7) *the predictability or unpredictability of the art*: The art is highly unpredictable as between hard pines and soft pines as established by the Rule 132 Declarations previously submitted. The art is also unpredictable as between regeneration of hard pines and regeneration of transformed hard pines. The specification describes a process for regenerating transformed hard pines that is predictable as between members of the hard pines as demonstrated by the working examples.

(8) *the breadth of the claims*: The breadth of the claims is to all pines of the genus *Pinus*, subgenus *Pinus*. This fact in and of itself does not render the claims non-enabled by the specification, especially in view of the extensive disclosure, extensive guidance and working

examples that show the regeneration of transformed hard pines, including Southern yellow pines. In view of these working examples and extensive guidance in the specification regeneration of transformed hard pines can be performed without undue experimentation.

It is clear from the above analysis of the Wands factors to the present invention that undue experimentation is not necessary in order to practice the claimed invention in view of the specific disclosure, working examples and guidance provided to a skilled artisan by the specification for practicing the claimed invention. This same analysis clearly demonstrates that the Examiner's contention that ABA is a required constituent for Applicants' method is incorrect. Thus, since the present application provides the skilled artisan with the details and guidance sufficient to make and use the claimed invention without undue experimentation, Applicants submit that the present specification enables the claimed invention.

In view of the above amendments and remarks, it is submitted that the specification fully enables the claimed subject matter. Withdrawal of this rejection is requested.

The Examiner rejected claims 39, 40, 42, 43 and 45 under 35 U.S.C. § 103(a) as being unpatentable over Levee et al. (*Molecular Breeding* 5:429-440, 1999) in view of Handley et al. It is submitted that the Examiner is in error in this rejection.

The subject matter of these claims is directed to a method for minimizing damage to transformed cells of pine of the genus *Pinus* subgenus *Pinus* following infection by *Agrobacterium*. The method comprises the steps of washing transformed cells in a liquid culture medium to minimize damage, plating the cells on a support membrane, suspending the cells in a liquid culture medium and recovering washed cells with minimal physical damage. Washing cells with a liquid culture medium is a positive step. The specification demonstrates that this method enables the recovery of transformed cells that can be regenerated into transgenic plants at an enhanced frequency. Applicants submit that this method is not disclosed or suggested by the cited prior art.

Levee et al. does not disclose a method to minimize damage to transformed cells of the subgenus *Pinus* which enables the transformed cells to be regenerated into transgenic plants. In fact, Levee et al. transforms *Pinus strobus*, a soft pine that is not a pine of the subgenus *Pinus*. Levee et

al. washed the transformed cells with three washes of sterile distilled water, one wash of a tetracycline solution and three washes of water. See page 431, right column. Levee et al. does not disclose the washing of transformed cells of the subgenus *Pinus* using a liquid culture medium as set forth in the claims. This step of washing transformed pine cells of the subgenus *Pinus* using a liquid culture medium is a positive step that distinguishes the claimed invention from the cited prior art.

Handley et al. discloses the regeneration of coniferous plants, which includes pines of the subgenus *Pinus*. Handley et al. does not disclose *Agrobacterium* transformation of the pines. Consequently, Handley et al. does not disclose washing transformed pine cells. Because Handley et al. does not disclose washing transformed pine cells, it does not teach or suggest using a liquid culture medium to wash transformed cells. This step of washing transformed pine cells of the subgenus *Pinus* using a liquid culture medium is a positive step that distinguishes the claimed invention from the cited prior art. Thus, the combination of Levee et al. and Handley et al. does not suggest washing transformed cells with a liquid culture medium that results in minimized damage to the transformed cells that in turn results in an enhanced frequency or regenerated transgenic plants. That is, none of the prior art alone or in combination suggests washing transformed pine cells of the genus *Pinus* subgenus *Pinus* with a liquid culture medium, a positive step set forth in the claims. Consequently, it is submitted that the claimed invention is not obvious from the cited prior art.

Furthermore, Levee et al. teaches transformation of soft pines and not pines of the subgenus *Pinus* (hard pines). Applicants have demonstrated in the previous amendments, the art cited by the Examiner and the submitted Rule 132 Declarations, that at the time of the present invention there were well known differences between soft pines and hard pines such that a skilled artisan could not reasonably predict whether process useful for one type of pine would work for the other type of pine. In view of these known differences the established unpredictability in the art and the failure of Levee et al. and Handley et al. to teach or suggest washing transformed pine cells of the subgenus *Pinus* with a liquid culture medium to minimize damage to the transformed pine cells, Applicants submit

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that the claimed subject matter of claims 39, 40, 42, 43 and 45 is not obvious from the prior art of record.

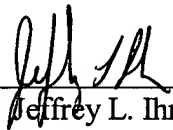
In view of the above amendments and remarks, it is submitted that the claimed invention is not obvious from the teachings of Levee et al. in view of Handley et al. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, and in conjunction with the remarks made in the previous amendments and previously filed Rule 132 Declarations, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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